

LCMS Identification of In Vitro Fertilization Culture Media Proteins

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Background and Significance

At the NYU Fertility Center 48% of cycles in women <35 years result in a live birth. Forty percent of these births in women <35 years at the NYU Fertility Center are twin deliveries. A strong criticism of assisted reproductive technologies (ART) is the high incidence of multiple gestations that increase fetal and maternal morbidity and mortality.

Grifo et al recently described their clinic's progression to blastocyst transfer as a means to reduce the high-order multiple rate.¹ The ART community has addressed the need for more single embryo transfers (SET) but also recognizes the lowered pregnancy rates that may ensue. The ability to identify additional markers associated with embryo viability and competence has been the greatest challenge towards promoting SET.

In a recent study of 3 and 5 day growth media, we have identified gi|223976 haptoglobin Hp2, mass 41717. Two more proteins were identified in our latest report, gi|90108928 1 Chain H, Orally Available Factor7a Inhibitor, mass 28582, and gi|119573737 hCG1793647 [Homo sapiens] Mass: 6112. This study produced two new specific biomarkers unique to competent embryos: gamma-aminobutyric-acid receptor subunit and tetatricopeptide repeat protein 9.

Materials and Methods

The IVF culture media from day 3 and day 5 embryos was extracted with organic solvent and high pressure using ProteoSolve© and the Barocycler© respectively (Pressure BioSciences, West Bridgewater, MA). Trypsin digest of the proteins was completed in 45 minutes, shortened from 12 hours. The peptide digest was studied with LCMS (Hitachi NanoFrontier nLC, Dallas, TX).

Results

The precursor ions were identified with MS. MSMS of the precursor ions generated a mass list. Matrix Science Mascot© software query of the NCBI nr data base with this mass list identified two novel proteins that were only present in the implanted embryos that resulted in live births: TTC9_HUMAN, Tetatricopeptide repeat protein 9 (TPR repeat protein 9) (Fragment) – Homo sapiens, Figure 1, and GBRE_HUMAN, Gamma-aminobutyric-acid receptor subunit epsilon precursor (GABA (A) receptor subunit epsilon) – Homo sapiens, Figure 2. They were not present in the poor quality embryos from the same patient.

Discussion

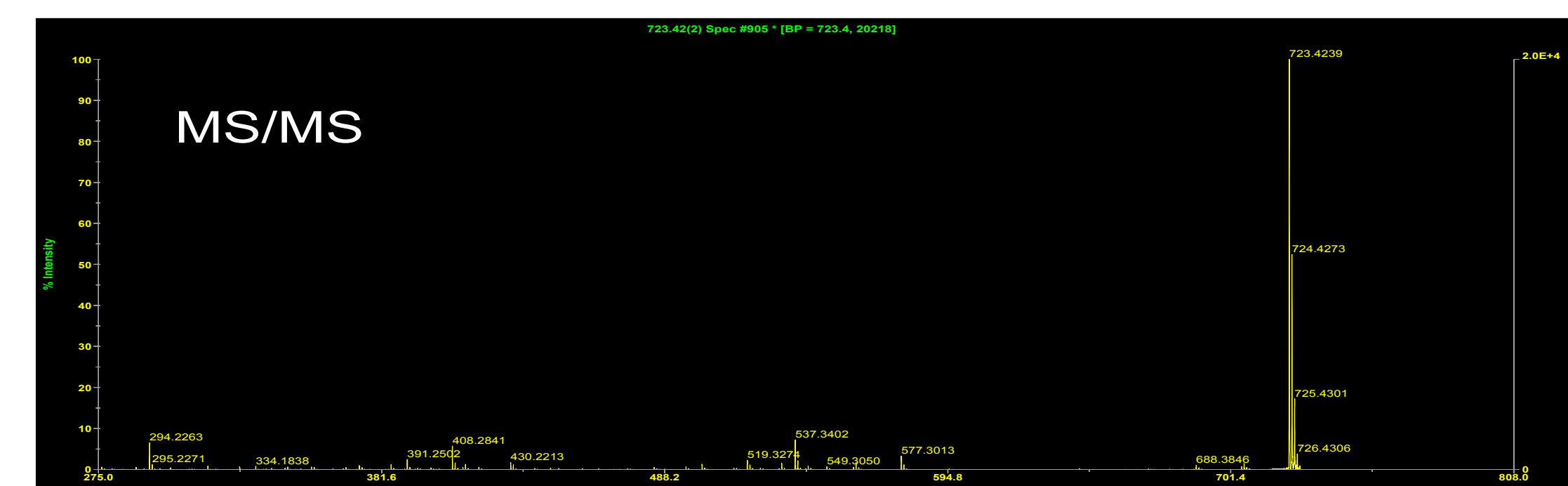
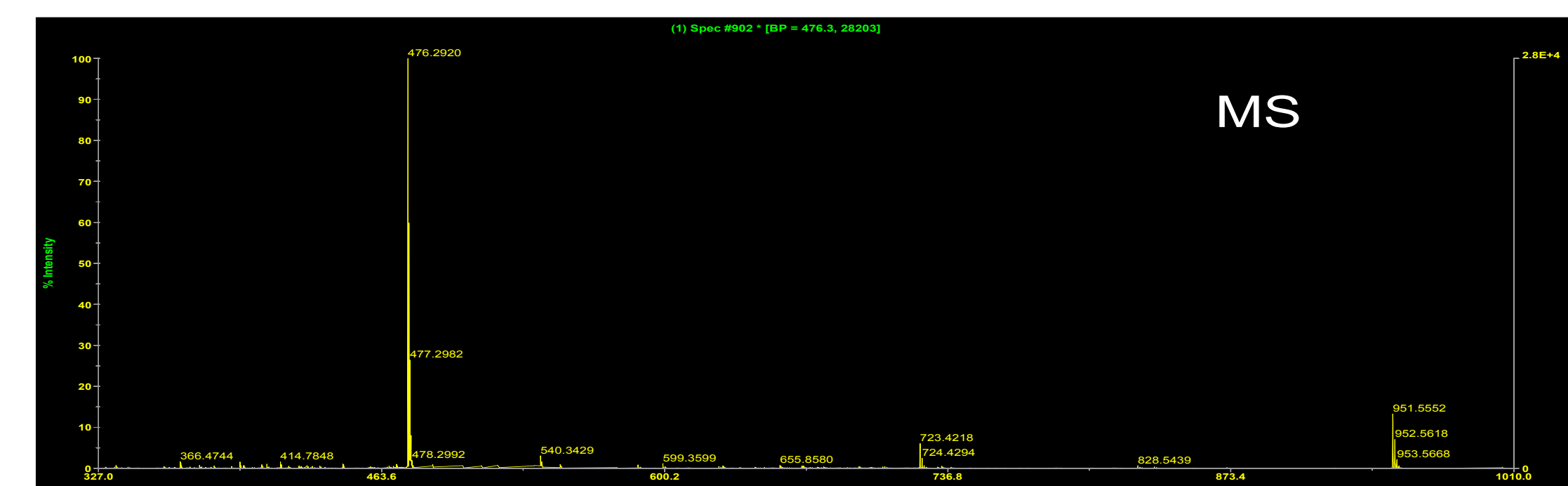
The Barocycler extraction resulted in a greater number of proteins identified than was noted in prior studies, and allowed a marked decrease in duration of trypsin digest from 12 hours to 45 minutes. Embryo quality is based on accepted microscopic morphologic criteria. However excellent morphology does not guarantee pregnancy. These new protein biomarkers of competent embryos should enhance embryo selection and improve live birth outcome with single embryo transfers.

REFERENCES

1. Grifo JA, Flisser E, Adler A, et al. Programmatic implementation of blastocyst transfer in a university-based in vitro fertilization clinic: Maximizing pregnancy rates and minimizing triplet rates. Fertil Steril. 2007;88:294-300.

Figure 1

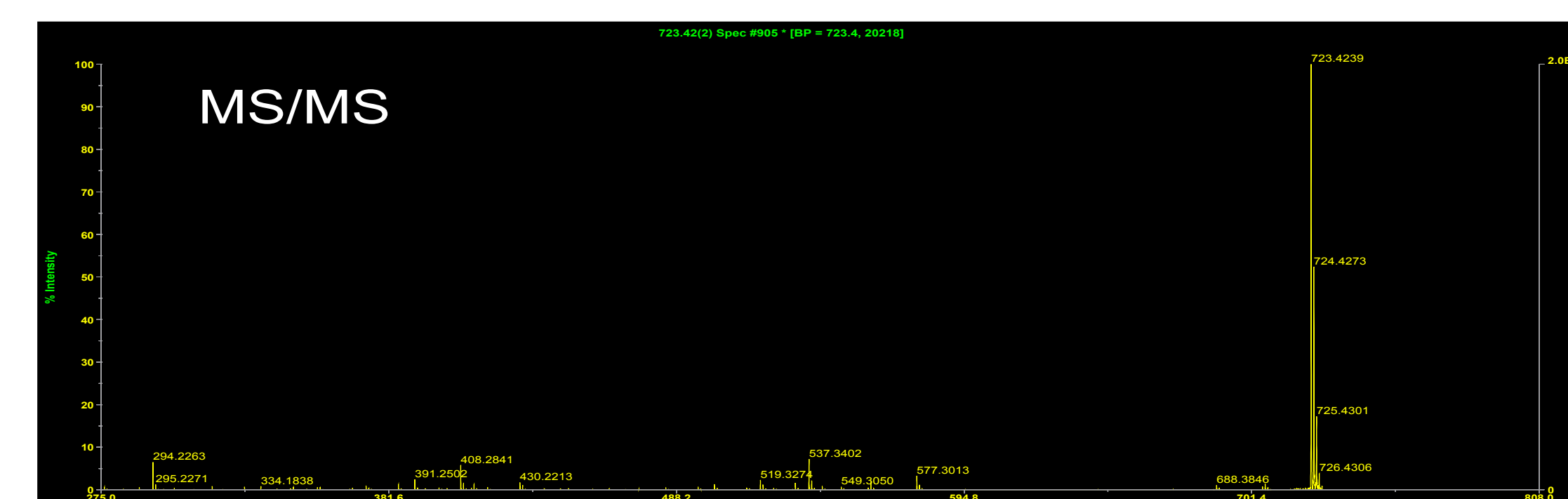
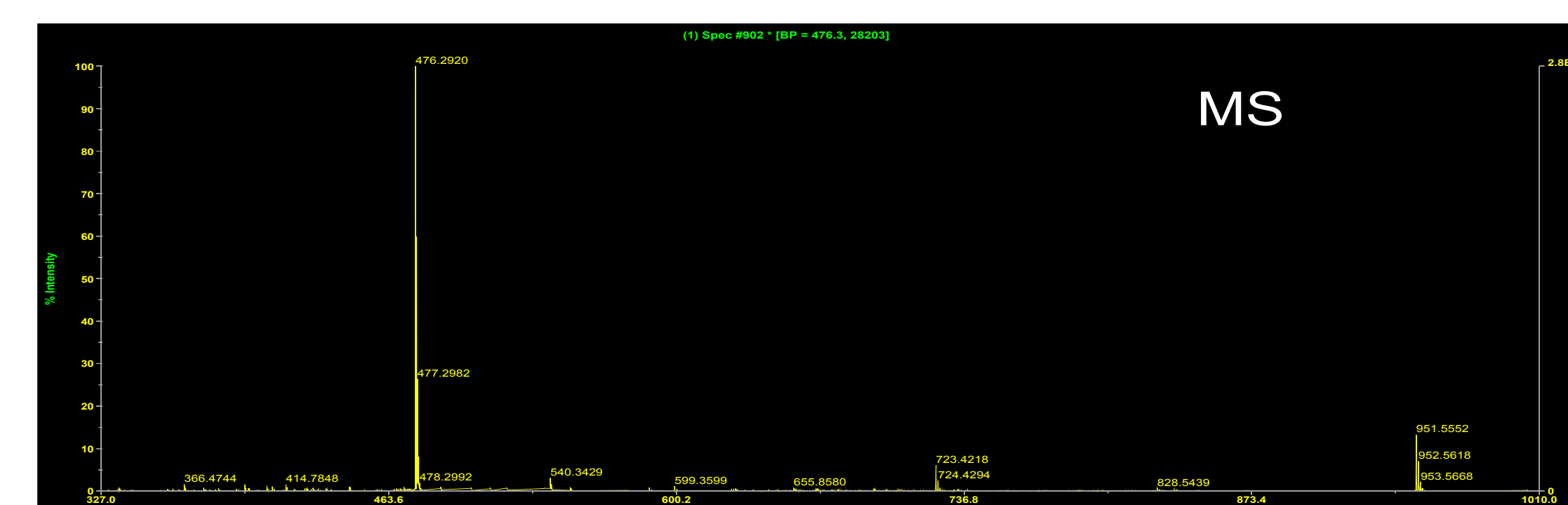
Sample: Sample 17: 10387A ER 6/26/05 #3 25
Protein: TTC9_HUMAN, Tetatricopeptide repeat protein 9 (TPR repeat protein 9) (Fragment) – Homo sapiens
Peptide Sequence: EGENFK
Mr(calc): 722.4150
MS/MS: 723.4223 (+1 Charge State)
Ions Score: 32
Expect: 0.032



**SAME ION DETECTED FOR GBRE_HUMAN

Figure 2

Sample: Sample 17: 10387A ER 6/26/05 #3 25
Protein: GBRE_HUMAN, Gamma-aminobutyric-acid receptor subunit epsilon precursor (GABA (A) receptor subunit epsilon) – Homo sapiens
Peptide Sequence: WENFK
Mr(calc): 722.3388
MS/MS: 723.4223 (+1 Charge State)
Ions Score: 32
Expect: 0.032



**SAME ION DETECTED FOR TTC9_HUMAN